Immunotherapy of Cancer and Autoimmune Inflammatory Using Redirected Lymphocytes and Prostate Cancer Diversity

Department of
Immunology

Prof. Zelig Eshhar

Tova Waks,
Dinorah Friedmann-Morvinski,
Lilach Agemy, Vica Malina,
Itai Kela, Charlott Servais,
Eran Elinav, Amit Maliar,
Assaf Marcus, Anat Globerson,
Dan Blat, Liat Elboim, Nitzan Adam

☎ 972 8 934 3965
FAX 972 8 9474 030
@ zelig.eshhar@weizmann.ac.il
www.weizmann.ac.il

T cells are the major arm of the immune system that recognize, discriminate, regulate and efficiently eradicate both large tumors as well as foreign or infected invaders. In certain disorders, however, effector T cells (Teff) that usually do not respond against the body's own constituents, respond against self and cause autoimmune diseases. Another sub-population, regulatory T cells (Treg), suppress Teff cells and keep them in check to prevent the burst of autoimmunity. Similar Treg however, may cause a problem when they are induced in the tumor vicinity and serve as part of the mechanisms that spontaneous malignancies develop to escape and evade Teff attack. The second arm of the immune response, tumor-specific antibodies, which can be more readily prepared and used for adoptive therapy, are less effective than T cells in the elimination of solid tumors. To overcome the rarity of tumor-specific T cells and inefficient rejection of solid tumors by antibodies, we developed the "T-body" approach in which we genetically redirect the recognition of T cells, using chimeric receptors genes whose recognition unit is made of an antibody binding site. Thus combining together the advantages of the cellular and humoral arms of the immune response for the immunotherapy of cancer. In studies performed in animal models for human breast and prostate cancers, we have provided a proof-of-concept for the "T-body" approach. More recently we have used the T body approach to redirect Treg to suppress accute inflammatory autoimmune colitis in mice. In order to study the variability of prostate cancer pathophysiology, the various human xenografts which we established and the TRAMP mouse model have been used.

Redirecting effector T-cells lymphocytes for cancer therapy

The most useful configuration of the chimeric receptor we use is the tripartite chimeric receptor (TPCR) that contains a scFv of a given antibody, the extracellular hinge, transmembrane stretch and cytoplasmic domains of CD28 linked to either the FcRγ or the CD3ζ stimulating regions. Such TPCRs induce full activation of T cells. Naïve, unprimed T cells of TPCR-transgenic mice undergo non-MHC restricted and co-stimulatory ligand independent stimulation for proliferation, high cytokine production and rescue from apoptosis upon encountering their antigen as well as specific activation and killing of target cells. To determine the clinical applicability of the 'T-Body' approach we have used erbB2 as the target antigen of choice. Expression of erbB2-specific TPCR in human T and NK cells using retrovectors yielded fully reactive T-bodies against tumor targets over-expressing erbB2 both *in-vitro* and *in-vivo*. Complete antitumor responses have been manifested in different regimens of T-body administration to SCID mice: direct intratumoral or systemic administration to orthotopically transplanted human breast and prostate cancer xenografts, systemic administration to disseminated lung-seeking metastases or established bone lesions. In all cases, a mild lympho-reductive pretreatments and post treatment administration of IL-2 were instrumental to enhance the antitumor response. To further optimize the *in-vivo* performance of the T-bodies towards their clinical application, we are testing their antitumor activity against mammary tumors that spontaneously develop in mice and express human erbB2.

Redirecting regulatory T cells to suppress autoimmune inflammation

Regulatory T cells (Treg) are known for their ability to suppress inflammatory responses; however, the scarcity of antigen-specific Treg impedes their clinical application. To study whether Tregs can be genetically modified to express non-MHC restricted chimeric receptor and maintain their suppressive function, we tested the ability of "Treg-T bodies" derived from TPCR transgenic mice to alleviate colitis. In models of acute colitis we have succeeded to demonstrate that isolated $CD4^+CD25^+Foxp3^+$ Treg expressing TNP-specific TPCR could specifically suppress the *in-vitro* activity of TNP-specific Teff cells. Furthermore, we found that adoptive transfer of small numbers of such Treg could both protect, alleviate and in fact rescue mice from TNBS (but not from oxazolone) induced colitis (Figure A). The "Treg-T bodies" migrated to and accumulated in the inflamed colon where they underwent specific stimulation to secrete their suppressive cytokines. At the site, these cytokines (e.g. TGFβ and IL-10) suppress inflammation in non-antigen specific manner. Thus, we have demonstrated a bystander effect in which Treg redirected to non-pathogenic antigen could be attracted to another antigen in the colon and suppress ongoing colitis. These encouraging results are now expended

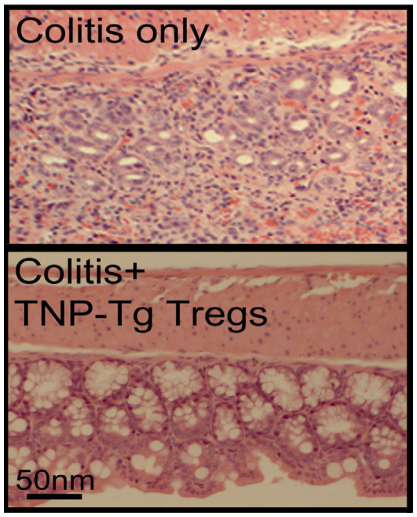

Fig. A *Histology of colon of a mouse with acute TNBS colitis (up) and after administration of TNP-specific redirected regulatory T cells (bottom). Note the heavily infiltrated colitic mucosa and normal appearance of the treated one.*

to other autoimmune conditions as well as other inflammatory conditions.

Diversity of prostate cancer progression and response to radiotherapy

Prostate cancer (PC) is a slow growing tumor that appears in adult men and is of diverse phenotype in its origin, progression, pathology and response to treatment. At its early stages, surgery, irradiation and androgen ablative therapies are quite effective and curative. However, at its advanced stages it is incurable. To have genetic insights into some aspects of this diversity, we used gene array analysis (with Prof. E. Domany, Physics of Complex Systems and Dr Alon Harmelin, Veterinary Resources). In one system, we studied transcription profiles in tissue and cancer specimens removed from the prostate gland and evolving adenocarcinoma at various stages from the TRAMP strain of transgenic male mice. This mice express the T-Ag under the probasin promoter that develope PC after puberty. The tumor progression in TRAMP mice mimics all stages of the human PC adenocarcinoma. We focused on comparing gene expression during different stages of tumor development: from normal to PIN to primary and disseminated PC. In parallel we compared the gene expression of two strains of TRAMP that develop different types of tumors with different aggressiveness. Finally, we compared the transcription profile of genes associated with PC development and progression in the TRAMP to existing databases of human PC and other cancers. In the search for genetic-background signature for outcome, we performed promoter analysis of the gene array data. One of the clusters that drew our attention is enriched with immune response related genes, which show higher expression levels in normal prostate of the less aggressive compared to the more aggressive TRAMP strain. Interestingly, the level of expression of these genes in the later strain went up during the premalignant PIN stage and decreased in the resulting cancer and metastases (Figure B). This led us to hypothesize

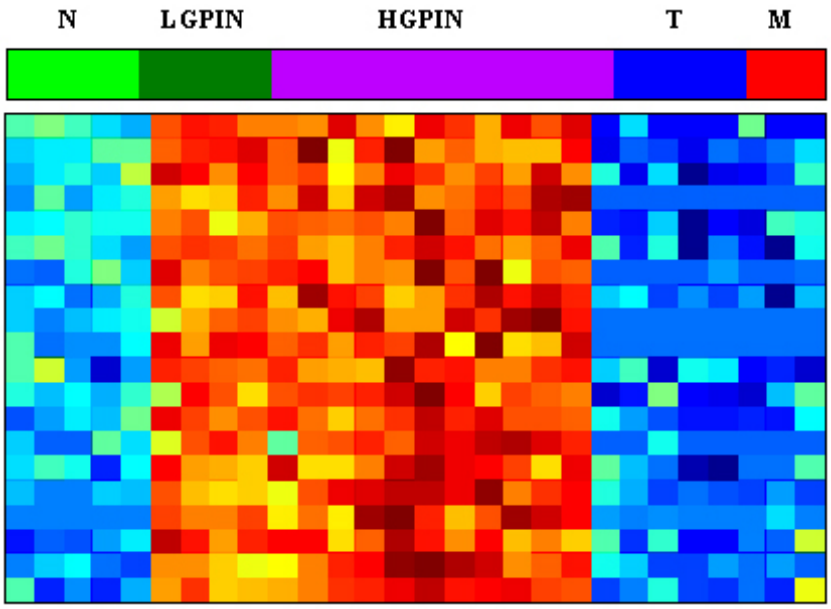

Fig. B *Gene expression array of the immune related-genes in different stages of development of prostate cancer in the TRAMP mouse. Tissue specimens (upper bar) are: N=Normal prostate; LGPIN and HGPIN= are low and high grade PIN (premalignant stages of PC); T-tumor; M=metastases*

that small but distinct differences in the basal activity of the immune system may play a role in development of the TRAMP prostate cancer.

In the second system, we took advantage of a collection of several PC xenografts that we have established (together with Prof. J. Ramon Sheba Medical Center and Dr I. Leibovich, Meir Hospital) in order to determine whether there is a genetic disposition in PC for resistance to irradiation. Ionizing radiation is the most common treatment used to control localized primary PC. However, for a significant number of patients, radiotherapy fails to adequately control the tumor. Thus, a main clinical problem today is the lack of specific markers to predict at diagnosis the treatment outcome and to identify patients who are unlikely to benefit from radiotherapy. We determined the radio-sensitivity/resistance of the PC xenografts to radiation and using expression microarrays, correlated their specific transcriptional profiles with response to radiation. Altogether, we have identified four gene clusters displaying different expression patterns. Two clusters showed higher expression levels in the resistant xenografts and the other two clusters showed higher expression levels in the sensitive xenografts. Expression levels of 113 genes differed at least 3 fold between sensitive and resistant xenografts. Selected genes from these clusters may be useful markers to enable prediction of the response of patients to radiotherapy. Interestingly, irradiation of the PC xenografts did not induce any significant changes in gene expression, regardless of their susceptibility phenotype. These data strongly support a model of a random effect of irradiation on a homogeneous population of cells, rather than that the PC is comprised of a mixture of radioresistant and radiosensitive cell subpopulations.

Selected publications

Pinthus, J. H., T. Waks, D. G. Schindler, A. Harmelin, J. W. Said, A. Belldegrun, J. Ramon, and Z. Eshhar. (2000). WISH-PC2: a unique xenograft model of human prostatic small cell carcinoma. Cancer Res 60:6563.

Willemsen, R. A., M. E. Weijtens, C. Ronteltap, Z. Eshhar, J. W. Gratama, P. Chames, and R. L. Bolhuis. 2000. Grafting primary human T

lymphocytes with cancer-specific chimeric single chain and two chain TCR. Gene Ther 7:1369.

Eshhar, Z., T. Waks, A. Bendavid, and D. G. Schindler. 2001. Functional expression of chimeric receptor genes in human T cells. J Immunol Methods 248:67.

Pinthus, J.H., T. Waks, K. Kaufman-Francis, D.G. Schindler, A. Harmelin, H. Kanety, J. Ramon and Z. Eshhar. 2003. Immuno-gene therapy of established prostate cancer using chimeric receptor redirected human lymphocytes. Cancer Res. 15: 2470-6

Pinthus,, J. H.,Waks, T., Malina V., Kaufman-Francis, K., Harmelin, A., Eisenberg I., Kanety, H., Ramon, J. and . Eshhar, Z. 2004. Adoptive Immunotherapy of Prostate Cancer Bone Lesions Using Redirected Effector Lymphocytes. J. Clinic. Invest. 114: 1774-1781.

Morvinski-Friedman D, A. Bendavid, T. Waks, D. Schindler and Z. Eshhar. 2005. Full activation of naïve T cells of transgenic mice expressing a tripartite chimeric receptor combining both stimulatory and co-stimulatory domains. Blood, 105: 3087-3093.

Eshhar,Z. Waks, T., Malina V. and Pinthus, J. 2005. Redirecting Immune Cells Against Bone Metastases: Immunotherapy of Prostate Cancer Metastases Using Genetically Programmed Immune Effector Cells. Discovery Medicine, Volume 5, Number 27, Pages 259-264.

Kershaw M.H.,Westwood J.A., Parker L.L.,Wang G., Eshhar Z., et al. (2006). A phase I trial utilizing adoptive transfer of gene-modified T cell for ovarian cancer. Clin. Can. Res, 12: 6106-6115.

Eshhar Z (2007) The T-body approach: redirecting T cells with antibody specificity. In: Nissim A and Chernajovsky Y (eds), Therapeutic antibodies, Springer, Berlin Heidelberg New York, pp 329–342

Agemy L., Harmelin A., Waks T., Leibovitch I., Rabin T., Pfeffer RM., and Eshhar Z (2008) Irradiation Enhances the Metastatic Potential of Prostatic Small Cell Carcinoma Xenografts. The Prostate, 68:530-539.

Eran Elinav, Tova Waks, Zelig Eshhar, Redirection of regulatory T cells with antibody specificity for treatment of colitis. In press, 2008

Acknowledgements

These studies were performed in part by the USA Army BCRP and PCRP, The Israeli Science Fund, The Prostate Cancer Foundation and the EC FP6 ATTACK program. ZE is an incumbent of the Renette and Marshal Ezralow Chair of Chemical and Cellular Immunology

INTERNAL support

ZE is an incumbent of the Renette and Marshal Ezralow Chail of Chemical and Cellular Immunology. Moross Institute of Cancer, Wolfson Center for Cancer Diversity; The Weizmann Institute –TLV Sourasky Medical Center Foundation; The estate of Irene Kuhn and Lotte Stern UK; and a grant from Marion Achtetuch Fund.